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Characterisation of Rotaviruses From Children Treated at a London Hospital During 1996: Emergence of Strains G9P2A[6] and G3P2A[6]

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Rotavirus strains from 171 patients treated in 1996 at a children's hospital in London were characterised. Use of a panel of typing monoclonal antibodies for serotypes G1–4 identified 105 (61%) of the strains. The majority, 90 strains (86%), were serotype G1. Characterisation of G (VP7) and P (VP4) types using reverse transcription–polymerase chain reaction was more efficient, and 167 of 171 (98%) of the strains were identified this way. The predominant strains were G1P1A[8] (55%) and G4P1A[8] (17%), which are prevalent throughout the world; however, a significant number of cases were associated with genotypes not recorded previously in the United Kingdom. There were 21 (13%) cases associated with G9P2A[6] and 11 (6%) cases associated with G3P2A[6] . The majority (seven of 10) cases of infection in children older than 3 years of age were caused by these two genotypes. A majority (15/21) of G9P2A[6] strains were recovered from children admitted to the hospital, and five children were sufficiently dehydrated to necessitate intravenous rehydration. ***J. Med. Virol. 61:150–154, 2000.***

KEY WORDS: rotavirus; genotype; serotype; VP4; VP7

INTRODUCTION

Group A rotaviruses are a leading cause of morbidity in young children and the elderly in the United Kingdom, with a peak incidence of infection between March and April [CDSC, 1998]. Extensive surveys were carried out in two regions of England, Birmingham [Beards et al., 1989] and London [Noel et al., 1991, 1994], to determine the temporal and geographical distribution of different G serotypes over the period 1983–1992. These studies showed that G1, G2, G3, and G4 were circulating in both communities throughout the period, but the relative prevalence of a particular serotype varied from season to season and between the two locations. A similar study in Melbourne covering the period 1973–1988 showed an almost identical situation [Bishop, 1994]. A problem with many surveys has been the inability to type a significant proportion of the rotavirus samples using limited panels of monoclonal antibodies (MAbs) [Beards et al., 1989; Bishop et al., 1989; Matson et al., 1990; White et al., 1991].

Information on the prevalence of P types circulating in the United Kingdom has been lacking, because reagents for serologic typing were not readily available and because there were some concerns about their specificity [Coulson, 1996]. The introduction of molecular techniques for determining G (for the VP7 protein) [Gouvea et al., 1990] and P (for the VP4 protein) [Gentsch et al., 1992] genotypes of rotaviruses permitted a more detailed characterisation of strains circulating in London during 1996 and an extension of our surveillance of rotavirus cases treated at Queen Elizabeth Hospital for Children (QEH) [Noel et al., 1991, 1994].

MATERIALS AND METHODS

During the period January to December 1996, stool samples from 199 patients treated for symptoms of diarrhoea and/or vomiting at the QEH were found to be rotavirus positive by electron microscopy. From 171 (86%) cases, sufficient faecal samples had been stored at 4°C to permit further characterisation of the viruses. The date of collection and the patient's age, sex, and hospital ward were available on computer databases. Further clinical information on patients infected with G9P6 rotavirus strains were obtained from the medical record.

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Enzyme Immunoassay for Determining G Serotype

Enzyme immunoassays (EIAs) were carried out as described previously [Noel et al., 1991] but using a different set of G-typing MAbs—KU-4 (G1), S2-G10 (G2), YO-1E2 (G3), and ST-2G7 (G4) [Taniguchi et al., 1987]—at a dilution of 1:10,000. Samples that failed to type were subgrouped using MAbs 255/60 (subgroup I) and 631/9 (subgroup II) [Beards et al., 1989]. Samples identified as G9 by genotype (see later discussion) were tested in an EIA as described previously herein but using MAb 45:8, which is specific for G9 rotaviruses obtained from E. Palombo, Royal Children's Hospital, Melbourne, Australia.

RNA Extraction and Genotyping

Viral RNA was extracted from faecal samples that had been stored at 4°C using TRIZOL (Gibco BRL, Scotland), as described previously [Cubitt et al., 1999]. Genotyping was undertaken by reverse transcription–polymerase chain (RT-PCR) reaction using primers Con2 and Con3 for gene 4 (VP4) [Gentsch et al., 1992] and Beg9 and End9 for gene 9 (VP7) [Gouvea et al., 1990]. The cDNA then was used for P type (VP4)–specific PCR using primers for P1A[8], IT-1; P1B[4], 2T-1; P2A[6], 3T-1; P3[9], 4T-1; and P4[10], 5T-1 [Gentsch et al., 1992] or G type (VP7)–specific PCR using primers for G1, aBT1; G2, aCT2; G3, aET3; G4, aDT4; G8, aAT8; G9, aFT9; and the common primer RVG9 [Gouvea et al., 1990]. The PCR products were analysed by agarose gel electrophoresis and assigned a genotype according to the size of the amplified product when run alongside a 100-bp DNA ladder (G2101; Promega, England).

Direct Sequencing of VP7 and VP4

Sequencing of VP7 and VP4 products initially was carried out manually at MEDUNSA; subsequent sequencing was accomplished using an automated sequencer.

RESULTS

Comparison of Reverse Transcription–Polymerase Chain Reaction and Enzyme Immunoassay for Typing Rotavirus Strains

The G type was obtained for 105 of 171 (61%) cases of rotavirus using typing MAbs [Taniguchi et al., 1987] against VP7. The majority—90 cases—were G1, followed by G4 (14 cases), G3 (two cases), and G2 (one case). Subgrouping (VP6) by EIA of the 66 untyped viruses showed that 22 were subgroup I and 17 were subgroup II; 27 failed to react.

The results of genotyping of VP7 and VP4 are shown in Table I. A total of 167 of 171 (98%) strains were characterised. The predominant genotypes were G1P1A[8] (92/167, or 55%) and G4P1A[8] (28/167, or17%) . Rotavirus type G9P2A[6], previously unreported in the United Kingdom, was detected in 21 of 167 (13%) patients;19 of these cases had been shown by EIA to be VP6 (subgroup I). Their identity was confirmed by sequencing the VP7 and VP4 genes and by reaction with G9 (VP7)–specific MAb 45.8. The VP7 sequences of G9 viruses obtained throughout the year were identical or differed by one or two amino acids, forming a tight cluster and suggesting that the strain was circulating widely in the community during 1996. The VP7 sequence showed 87% nucleotide and 92% amino acid homology with G9 strain 116E [Iturriza et al., unpublished observations], found in asymptomatic neonates [Das et al., 1993]. Eleven cases of infection with G3P2A [6] were identified, only one by EIA, using Mab YO-IE2, whereas both cases of G3P1A[8] were detected with this MAb. Dual infection with G1 and G4 was recognised in two individuals; all these cases would have been described as G1 cases using EIA. Six samples (one, G1; one, G3; four, G4) yielded a first-round product obtained with the VP4 (gene 9)–specific primers, but no product was obtained in the second-round reaction.

TABLE I. Results of Genotyping Rotavirus Samples From 171 Patients*

	P type (%)				
G type	P1A[8]	P1B[4]	P2A[6]	Uncertain[a]	Total
G1	90 (53)		2 (1)	1 (0.6)	93 (55)
G2		5(3)			5 (3)
G3	2 (1)		11 (6)	1 (0.6)	14 (8)
G4	26 (16)			4 (2)	30 (18)
G9			21 (12)		21 (12)
G1 + G4	2 (1)				2 (1)
G uncertain[b]		2 (1)			2 (1)
Total	120 (71)	7 (4)	34 (19)	6 (4)	167 (98)

*Four samples could not be characterised by genotyping or serotyping.
[a]Polymerase chain reaction product detected only in first round of amplification.
[b]G genotype not obtained.

Seasonal Distribution of Rotavirus Genotypes

The seasonal distribution of the four most common genotypes that were found during 1996 is shown in Fig. 1. The rotavirus season in 1995–1996 started later (in December) and lasted longer (until August) than in previous years. In November 1995, no rotavirus cases were detected, and only four cases were identified in December. The peak of infection occurred in March 1996, coinciding with the peak incidence of cases associated with G1P1A[8]. In contrast, the peak incidence of G9P2A[6] cases was late in the season (May), at a time when the number of cases of G1 and G4 infections was declining. There were insufficient cases associated with G2 and G3 to show a seasonal distribution.

Age Distribution of Rotavirus Infections

The distribution of cases by age and genotype is shown in Fig. 2. The peak incidence of rotavirus infections associated with G1P1A[8] and G4P1A[8] occurred in children between 7 months and 2 years of age. Eight cases of rotavirus infection were documented in chil-

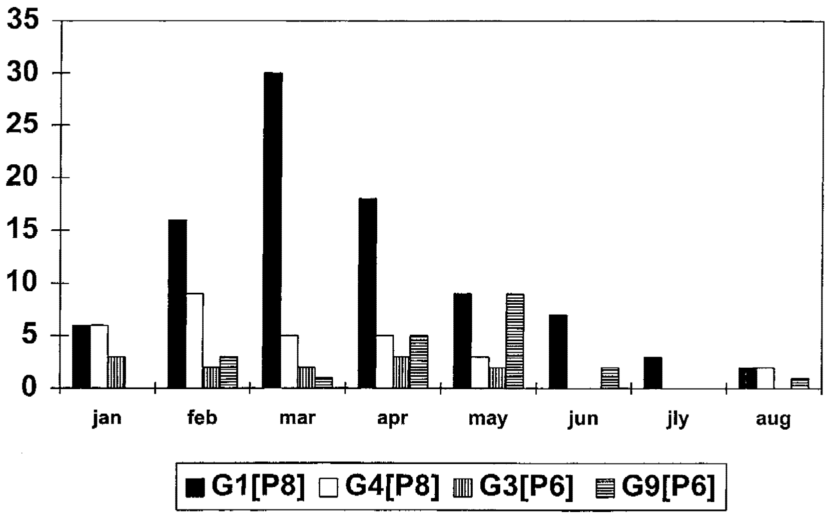

Fig. 1. Seasonal distribution of the four most common genotypes of rotavirus in 1996. Note the peak incidence of infection with G1 at the usual time March, whereas the peak incidence of G9 cases occurred in May.

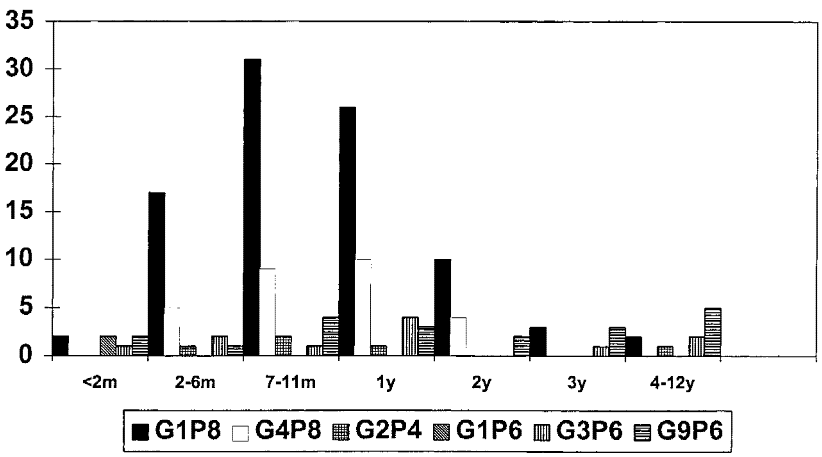

Fig. 2. Age distribution of rotavirus cases according to genotypes. Note that the majority of cases in children in the age groups <2 months and 4–12 years were associated with P2A[6] rotavirus strains.

dren aged < 2 months old; five were associated with P2A[6], two with P1A[8], and one with a G1 strain in which only a first-round product was obtained in the P-typing RT-PCR. Only 10 of 199 (5%) cases of rotavirus occurred in children > 3 years old; seven (70%) were associated with P2A[6] strains (two were G3, and five were G9). The oldest child to be infected with G9P2A[6] was 12 years of age.

Features of Infection With G9P2A[6]

The predominant symptoms of infection with G9P2A[6] were diarrhoea and vomiting; 15 of 21 patients (71%) were sufficiently unwell to be admitted to the hospital rather than being treated in the Casualty Unit, given advice, and discharged home. Details of therapy were available for 15 of the patients, five (33%) of whom were severely dehydrated and required intravenous fluids. Only two cases of infection were likely to have been acquired nosocomially. The first patient, an infant who was 5 months of age, was admitted 9 days before the onset of diarrhoea with atypical eczema and feeding problems. The second case was in a neonate, who had been in the hospital for more than a week before virus was detected; no other cases of rotavirus were identified in this ward in the 2-week period before her infection.

Patients with G9P2A[6] infection resided in seven different postal districts and were treated during a 7-month period, indicating that many of the cases were unrelated and suggesting that the strain of rotavirus was circulating widely throughout the East End of London during 1996. Secondary cases of infection were detected in two families. In one family a 2-year-old boy was admitted to the hospital with severe diarrhoea 2 days after his brother, aged 10 months, had been admitted. The other related cases were a 7-year-old boy and his 1-year-old sister, who showed symptoms a day after her brother.

Ethnic Group of Patients Infected With G9P2A[6]or G3P2A[6] Strains

G9P2A[6]. Judging by the patient's family names, 11 of the affected children were likely European, eight were African, and two were Asian. The majority of patients (four of five) requiring intravenous fluids were European.

G3P2A[6]. Six of the patients had names of African origin, four were Europeans, and one was Vietnamese.

DISCUSSION

We used EIA to monitor the prevalence of rotavirus G types among patients treated at QEH over the period 1984–1992 [Noel et al., 1991,1994]. This investigation showed that the predominant virus type was G1 throughout the study period, except for 1989–1990, when G4 accounted for 56% of cases. In the present study, which covered the period January to December 1996, G1P1A[8] was the predominant genotype, accounting for 55% of the cases, followed by G4P1A[8] (15%) and G9P2A[6] (13%).

In earlier studies we used MAbs produced by Coulson et al. [1987] for G serotyping G1–4 and G8 and identified 668 of 781 (86%) of the isolates [Noel et al., 1991]. In contrast, in the present study using MAbs to G1–4 produced by Taniguchi et al. [1987], only 105 of 171 (61%) of cases were distinguished. Nonetheless, 21 cases (13%) were found to be G9. The use of RT-PCR to genotype the rotaviruses was found to be more efficient than MAb-based EIAs, resulting in identification of 167 of 171 (98%) of the samples. It was particularly noticeable in this study that the MAbs raised by Taniguchi et al. [1987] failed to detect the majority of rotaviruses of types other than G1P1A[8].

Identification of the P types of rotaviruses circulating in London has not been reported previously, owing to concerns about the specificity and validity of typing samples using MAbs raised against VP4 [Coulson, 1996]. Application of RT-PCR allows for rapid identification of the P type and proved very effective even on samples that had been stored for several years at 4°C. G1P1A[8] and G4P1A[8] genotypes predominated, as in other studies in developed regions of the world [Gentsch et al., 1996].

In the present study (Table I) a large number of cases (35/167, or 21%) were associated with P2A[6] genotypes: 21 cases of G9, 11 cases of G3, two cases of G1, and one case of G4. G9P2A[6] strains have been reported to be a common cause of diarrhoea in India [Ramanchandran et al., 1996], but these are the first cases to be documented in England. All the G9P2A[6] cases were subgroup I, and sequence data of the VP7 gene of several of the viruses indicated that they share a high degree of homology (91%) with Indian isolate 116E, characterized by Das et al. [1993] . The use of MAb 45.8 showed them to be antigenically related to other G9 strains. Although MAbs to G9 were not available for our earlier study, it is unlikely that G9P2A[6] strains were circulating in east London during 1984–1992, since all strains found to be subgroup I were identified as G2. It is therefore probable that G9P2A[6] strains have entered the population since 1992. A recent survey in Bangladesh suggests that G9P2A[6] strains emerged there in 1995 [Unicomb et al., 1999]. The East End of London is a multiracial community with large numbers of immigrants from the Indian subcontinent and Africa. It was speculated that G9 strains may have been introduced as a result of frequent travel between India and Bangladesh, where G9P2A[6] infections are common; however, the ethnic origin of the affected patients was predominantly Caucasian or African, raising the possibility that G9P2A[6] strains are present in Africa. This theory is supported by a recent study of rotavirus isolates collected in 1997–1998 in Malawi, where 3% of cases were identified as G9P2A[6] [Cunliffe et al., 1999].

It is of interest that G9P2A[6] cases were as common in children older than 2 years as in younger children, which is unlike the pattern seen for G1P1A[8] and G4P1A[8] infections, which had a peak incidence in the age group 7 months to 2 years. These findings suggest that previous infection with the common genotypes of rotavirus, G1P1A[8], G4P1A[8] and G2P1B[4], fails to afford protection against severe illness associated with G9P2A[6] . Data were available on the therapy given to some of the children who were infected with G9P2A[6]; five of 15 (33%) required intravenous rehydration, which contrasts with findings of our previous study, in which only 15 of 230 patients infected with G1 strains were given intravenous infusions [Noel et al., 1994].

Infections due to rotavirus G3 are not uncommon in the United Kingdom [Beards et al. 1989; Noel et al., 1991, 1994], but recent studies have shown that the majority of cases are G3P1A[8] [Iturriza and Gray, personal communication]. In contrast, in this study, the majority of G3 strains were shown to be G3P2A[6], a genotype not previously reported in the United Kingdom but found to account for 10% of cases in Malawi in 1997–1998 [Cunliffe et al., 1999]. The results of the present study raise several concerns. Are rotavirus G9P2A[6] and G3P2A[6] strains emerging as an important agent of diarrhoeal disease? The data presented here and in recent reports from the United States and Bangladesh suggest that this may be the case for G9P2A[6] [Kirkwood et al., 1999]. Why is previous exposure to rotavirus infections apparently failing to prevent severe illness in older children? One explanation is that the virus is infecting a virgin population, but it is highly improbable that older children would never have been exposed to rotavirus infection. An alternative explanation is that the VP4 component is important and that the severity of infection was related to lack of immunity to P2A[6] strains, which are believed to be rare in the United Kingdom [Gray et al., personal communication]. Nevertheless, the G3P2A[6] infections were not as severe as G9P2A[6] infections, raising the possibility that the recently introduced tetravalent vaccine, which does not contain a G9 component, may not confer immunity to G9P2A[6] infection and that vaccination may open up an ecological niche for it to emerge more rapidly. Further characterisation of the G9 and G3 strains is in progress, and retrospective surveillance of rotavirus infections at QEH should enable us to establish when G9P2A[6] first emerged in the community.

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